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JACOB MENSAH-ATTIPOE

MICROBIAL CONTAMINATION OF BUILDING MATERIALS – GROWTH AND AEROSOLIZATION

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ABSTRACT

Moisture-damaged building materials promote microbial growth and become sources of microbial contamination in indoor environments. These contaminants, such as fungal spores and fragments, have been claimed to cause adverse health effects in the occupants of these buildings. The aim of this thesis was to evaluate the susceptibility of building materials to fungal growth and to measure and characterize the fungal spores and fragments being aerosolized from surfaces contaminated with fungi.

Five different analysis methods were used to assess temporal variations of fungal growth on two classes of building materials, so-called green and conventional. The concentration and properties of fungal spores and fragments aerosolized from the material surfaces were determined using optical particle counters and laser induced fluorescence devices as well as visualization in the scanning electron microscope coupled with energy dispersive X-ray spectroscopy.

The results showed that the chemical composition, nutritional value and moisture content of the building materials affected fungal growth; instead the classification of the materials into green or conventional categories exerted no influence. However, in the presence of dust, growth was seen on all of the materials irrespective of the chemical composition or nutritional value. Of the five methods, the cultivation method was most sensitive at revealing the temporal variations in the fungal concentrations, whereas the qPCR technique detected the highest biomass. Each assay method, however, provided a different perspective of fungal quantification i.e. there were method specific responses to the different stages of fungal growth. The results also indicated that species of fungi, age of the culture, the types of growth substrates and the air velocity over the growth surface all affected the fluorescent properties of the aerosolized spores and the concentrations of spores and fragments. Fungal fragments were shown to be formed by mechanical processes and the detection of

nitrogen and phosphorus in aerosolized fragments proved be a good indicator of the biological origin.

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Jacob Mensah-Attipoe
Kuopio, January 2016

LIST OF ABBREVIATIONS

ABD – Acoustic board (green)
ABY – Acoustic board (Non-green)
APS – aerodynamic particle sizer
Aw – water activity
a_w – water activity
BWA – Biological warfare agents
CFU – colony forming units
d_a – aerodynamic diameter
DGGE – denaturing gradient gel electrophoresis
DNA – Deoxyribonuclease
DOA – dioctyladipate
DOP – dioctylphthalates
EDX – Energy Dispersive X-ray
EIA – Enzyme immunoassay
ELPI – electric low pressure impactor
EMC – equilibrium moisture content
ERMI – environmental relative mouldiness index
ESP – extracellular polysaccharide
F/S – fragment to spore ratio
FSSST – Fungal Spore Source Strength Tester
GC-MS – Gas Chromatography Mass Spectroscopy
IOM – Institute of Occupational Medicine
ITS – internal transcribed spacer
LAL – Limulus amebocyte lysate
LIF – laser induced fluorescence
LOD – Limit of Detection
MALDI-TOF-MS – Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry
MC – Moisture content
MCWA – microbial cell wall agents
MHC – Moisture Holding Capacity
N – Nitrogen
NAHA – N-acetylhexosaminidase
NGS – Next generation sequencing
ODTD – organic toxic dust disease

OPC – optical particle counter
P - Phosphorus
PBOA – primary biogenic organic aerosols
PM – Particulate Matter
POBA – primary organic biological agents
PVC – polyvinyl chloride
qPCR – quantitative Polymerase Chain Reaction
RH – Relative humidity
SEM – Scanning Electron Microscope
TEM – Transmission Electron Microscope
UVAPS – Ultraviolet aerodynamic particle sizer
VOCs – Volatile Organic Compounds
WHC – water holding capacity
WHO – World Health Organization
WIBS – Wide Issue Bioaerosol Sensor/Waveband Integrated Bioaerosol Sensor

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman numerals I–IV.

I Jacob Mensah-Attipoe, Tiina Reponen, Anniina Salmela, Anna-Maria Veijalainen, Pertti Pasanen. Susceptibility of green and conventional building materials to microbial growth. *Indoor Air 25: 273–284, 2015.*

II Jacob Mensah-Attipoe, Tiina Reponen, Anna-Maria Veijalainen, Helena Rintala, Martin Täubel, Panu Rantakokko, Jun Ying, Anne Hyvärinen, and Pertti Pasanen. Comparison of methods for assessing microbial growth on building materials. *Submitted, 2015.*

III Sampo Saari, Jacob Mensah-Attipoe, Tiina Reponen, Anna-Maria Veijalainen, Anniina Salmela, Pertti Pasanen, Jorma Keskinen. Effects of fungal species, cultivation time, growth substrate and air exposure velocity on the fluorescence properties of airborne fungal spores. *Indoor Air 25: 653–661, 2015.*

IV Jacob Mensah-Attipoe, SampoSaari, Anna-Maria Veijalainen, Pertti Pasanen, Jorma Keskinen, Jari T.T. Leskinen and Tiina Reponen. Release and characterization of fungal fragments in various conditions. *Accepted, Science of the Total Environment, 2015.*

AUTHOR'S CONTRIBUTION

Paper I Jacob Mensah-Attipoe contributed to the design of the study, conducted the laboratory work and analyzed the data. The author wrote the manuscripts with significant editorial input from all co-authors.

Paper II Jacob Mensah-Attipoe contributed to the design of the study, conducted the laboratory work, and analyzed the data. The author wrote the manuscripts with significant editorial input from all co-authors.

Paper III Jacob Mensah-Attipoe contributed to the design of the study and helped with the laboratory work. Sampo Saari analyzed the data and wrote the manuscript with assistance from co-authors.

Paper IV Jacob Mensah-Attipoe contributed to the design of the study, conducted the laboratory work, and analyzed the data. The author wrote the manuscript with significant editorial input from all co-authors.

Contents

1 General Introduction

1.1 BIOAEROSOLS IN INDOOR AIR

It is estimated that a human being, on average, inhales 10 m^3 of air every day and spends > 75% of time in indoor environments (Dacarro et al., 2003; Klepeis et al., 2001; Tringe et al., 2008). Thus, almost everyone is commonly exposed to indoor pollutants which can be considered to consist of bioaerosols, non-biological particles, gaseous components and volatile organic compounds (VOCs) (Nevalainen and Seuri, 2005; Norback et al., 2013; Sordillo et al., 2010).

Bioaerosols are one fraction of the total aerosol mass; they consist of particles that originate from biological materials or processes which have a biological origin, for example, bacteria, fungi, pollen, viruses and their fragments and by-products (e.g., endotoxin and mycotoxin) as well as particulate waste products or fragments from living organisms (e.g., animal allergens) (Douwes et al., 2003). These particles are a complex and highly variable mixture of elements that differ in terms of their biology, chemistry and morphology. Fungal spores are considered the most abundant fraction of these particles; they have an aerodynamic diameter (d_a) in the size range of 1 μm–10 μm (Glikson et al., 1995). Due to their abundant substrates (e.g., plants, soil, water, animals and human activities), fungal spores and fragments have been found to be one of the most common classes of airborne biological aerosols in many indoor environments and they form part of the complex community of indoor biological agents (Bauer et al., 2008; Dacarro et al., 2003; Elbert et al., 2007; Nevalainen and Seuri, 2005; Reponen et al., 2011; Womiloju et al., 2003). Therefore, it has been proposed that the detection of the indoor presence of particular fungi e.g., *Aspergillus, Penicillium* and *Alternaria,* can be used as an indicator

of indoor air quality (Andersen et al., 2011; Araujo et al., 2008; Vesper, 2007).

Indoor fungal exposures are receiving increasing attention as an occupational and public health problem; this is due to the high prevalence of fungal contamination in buildings. Dampness and moisture-related problems are the main sources of fungal contaminations (IOM, 2004; WHO, 2009). Homes and other domestic dwellings (Flannigan et al., 2002) as well as schools (Norback et al., 2013) suffering from dampness and moisture damage commonly reveal the presence of elevated concentrations of fungi in comparison to buildings with no such problems.

Statistics reveal that about 55% of Finnish residences are in need of remediation out of an estimated 45–80% of buildings that have moisture damage (Nevalainen et al., 1998; Ruotsalainen et al., 1995). However, the problem is not limited to Finland alone. It has been estimated that dampness and mould growth can be detected in 20–50% of US homes as reviewed by Mudarri and Fisk, (2007) and these have been associated with increases of 30-50% in several respiratory and asthma-related health outcomes (Fisk et al., 2007). Furthermore, approximately 8–18% of cases of acute bronchitis and 9–20% of respiratory infections are estimated to occur in environments contaminated with fungi (Fisk et al., 2010).

The review of Samson et al, (2010) claimed that floods, wet seasons, thermal modernization of residential buildings, air-conditioning systems, construction or material faults, and poor and improper ventilation were the major reasons for increase in the relative humidity and dampness of materials in the indoor environment. If moist conditions are prolonged in indoor environments e.g. if building materials stay damp for a long time, then the growth of microbes is promoted and there is an increased risk of microbial contamination (Piñar and Sterflinger, 2009; Samson et al., 2010; Sterflinger, 2010). In addition, certain characteristics of the home (Sordillo et al., 2010) as well as

personal activities of its occupants (Dunn et al., 2013) influence the microbial profile in indoor environments.

Generally, a wide range of fungal species may be encountered in the indoor air. For example, Zyska (2001) surveyed the available literature and compiled a list of more than 200 fungal species present in the air or growing on structural materials in indoor environments and therefore likely to contribute to the airborne fungal burden. Fungi in indoor environments can be inhaled and exposure via the airways is especially problematic. Furthermore, the presence of fungal bioaerosols has been linked to many diseases and symptoms among the occupants of moisture damage buildings (Gutarowska and Piotrowska, 2007; IOM, 2004; Samson et al. 2010; WHO, 2009). This thesis focuses on fungal growth on building materials and on the aerosolization of fungal particles into indoor environments.

1.2 HEALTH EFFECTS OF FUNGI IN INDOOR ENVIRONMENT

Although there is more and more public interest in the microbial components in indoor air, the sources of the microbial communities and the processes that affect them are not well understood (Corsi et al., 2012; WHO, 2009). Exposure to bioaerosols including fungi have been linked to a range of adverse health effects (Douwes and Pearce, 2003). For example, exposure to fungi has been associated with the onset of asthma in both infants and adults (Bornehag et al., 2001; Bornehag et al., 2004; Hope and Simon, 2007; Hope 2013; IOM, 2004; Jaakkola et al., 2005; Johanning et al., 2014; Johanning, 2004; Kanchongkittiphon et al., 2015; Karvala et al., 2010; Meggs, 2009; Mendell et al., 2011; Tischer and Heinrich, 2013; Tischer et al., 2011; WHO, 2009).

Studies to examine the association of health effects of fungi have used two basic methods i) qualitative observations, such as visible water damage, visible evidence of moisture damage and

fungal growth, fungal odor and water leaks (current or past) and ii) quantitative measurements of microbial concentrations such as counting total culturable fungi, specific culturable fungi, or total fungal spores, and measurements of chemical and biochemical components released by the fungi. These qualitative assessments have shown sufficient evidence of a causal link between observed mold and moisture damage and asthma exacerbation in children as reviewed by Kanchongkittiphon et al. (2015). In addition, there is convincing data in the literature that there is an association between moisture damage in a building and the incidence of diseases such as new asthma cases, current asthma, respiratory infections, cough, allergic rhinitis, eczema and bronchitis with various upper respiratory tract symptoms (Bornehag et al., 2001; Bornehag et al., 2004; Hope and Simon, 2007; Hope, 2013; IOM, 2004; Jaakkola et al., 2005; Johanning et al., 2014; Johanning, 2004; Kanchongkittiphon et al., 2015; Karvala et al., 2010; Meggs, 2009; Mendell et al., 2011; Tischer and Heinrich, 2013; Tischer et al., 2011; WHO, 2009). In contrast, quantitative assessments have not detected any consistent associations between fungal measurements and adverse health effects. Nevertheless, limited or sufficient associations have been documented between the fungal concentration in dust by qPCR, cultured airborne fungi sampled from indoor air as well as several microbial compounds such as ergosterol, endotoxins and beta-glucans in dust and adverse health effects (Biagini et al., 2006; Iossifova et al., 2009; Iossifova et al., 2007; Reponen et al., 2011; Reponen et al., 2012).

Based on the above quoted studies, there is credible scientific evidence to support the association between moisture damage, visible fungal growth measured indoors and adverse health effects. The World Health Organization (WHO) has stated that approximately 25% of residents in European social housing stocks are prone to experience elevated health risks associated with their exposure to indoor moulds and that this is responsible for an annual economic loss in terms of healthcare and sickness leave amounting to 5.8 billion euros (Bonnefoy et al., 2003). In addition, it has been estimated that approximately 4.6 million of

the current cases of asthma in the U.S. are attributable to dampness and mould exposure and that this poses an estimated economic burden of 3.5 billion dollars annually (Mudarri and Fisk, 2007).

The health effects associated with fungal exposures may be caused by the fungi themselves, fungal mycotoxins, and fungal cell wall components or metabolically produced volatile compounds (Korpi et al., 2009). The health effects can be categorized into three groups: 1) infections, which are caused mostly by the viable cells (Falvey and Streifel, 2007); 2) allergic reactions, which are usually caused by both viable and non-viable cells and components of the cell wall of the fungi if they carry antigens (Green et al., 2006; Green et al., 2003) and 3) toxic responses, usually in response to the mycotoxins produced by the fungi (Brasel et al., 2005).

In addition, non-specific symptoms such as eye, nose and throat irritation and fatigue have often been found in connection with building related problems (Hope and Simon, 2007; WHO, 2009). These symptoms disappear when the occupants leave the environments where the exposures occur. This type of symptomology is called "building-related symptoms" or "sick building syndrome" (SBS) (Burge, 2004). While the etiology of these symptoms is not fully understood, allergens from fungal growth have been considered to be one of the causes of health problems in buildings. For example, there are reports of IgE-mediated mould allergy leading to allergic rhinitis or asthma respiratory hypersensitivity as well as fungal infections occurring from exposure to high fungal concentrations (Crook and Burton, 2010). Therefore, it has been suggested that both allergic and non-allergic mechanisms are likely involved in the etiology of the adverse health effects (WHO, 2009).

Some microbial exposures, should they occur early in life, such as exposures to endotoxins and (1→3)-β-D-glucan (Douwes, 2005; Douwes et al., 2006; Iossifova et al., 2009; Iossifova et al., 2007;

Park et al., 2001; Schram-Bijkerk et al., 2006) are protective when an individual comes into contact with dogs, pigs, cows, chickens, feed and grains as well as diverse bacteria and fungi. It is known that exposure to these fungi and their components reduces the development of atopy as well as the appearance of asthma and sensitization to inhalant allergens in children.

These above studies were limited to infants; in contrast, Rylander et al. (1998) reported a positive correlation between increased airborne (1→3)-β-D-glucan and upper airway symptoms in atopic school children between the ages of 6 and 13 years. Likewise, Douwes et al. (2006) showed that increased levels of (1→3)-β-glucan in household dust displayed a positive relationship with the variability in peak expiratory flow in non-atopic children between the ages of 7 and 11 years. However, an inverse association between the (1→3)-β-D-glucan concentration in household dust and atopic wheeze has been detected in children between the ages of 5 and 13 years as well as in younger children (1 – 4 years) with both asthma and persistent wheeze (Douwes et al., 2006; Iossifova et al., 2009; Iossifova et al., 2007; Schram-Bijkerk et al., 2006). Furthermore, Iossifova et al. (2009) have reported that increased exposure to high (1→3)-β-D-glucan concentrations decreased the risk of wheezing.

Exposure to endotoxin has been shown to be associated with a slightly increased risk of wheezing in children with atopy (Bakolis et al., 2012; Iossifova et al., 2009; Park et al., 2001). However, an inverse association has been reported between increased endotoxin concentration and atopic wheeze (Schram-Bijkerk et al., 2006; van Strien et al., 2004). This association failed to remain statistically significant when both endotoxins and (1→3)-β-D-glucan were included in the deterministic models. This was most likely caused by a positive and significant correlation between the levels of endotoxin and (1→3)-β-D-glucan (Douwes et al., 2006; Schram-Bijkerk et al., 2006). Thus, quantified microbial exposures have not yet been consistently associated with adverse health effects.

An interesting paradox is evident in many studies which seem to suggest that a wide diversity of fungi together with other bacteria confer protection against certain diseases and symptoms for individuals living on farms (Ege et al. 2007; Genuneit, 2012). For example, it has been shown that more diverse microbial exposures may be protective against allergy and asthma (Ege et al., 2007; Ege et al., 2011; von Mutius and Radon, 2009). This is most clearly seen in infants, since the developing immune system benefits from being challenged with natural microbes (Heederik and von Mutius, 2012). Thus, farming children are less likely to suffer from allergies than their urban counterparts (Ege et al., 2011; Ege et al., 2007; von Mutius and Radon, 2008). In a more recent pilot-scale study, Dannemiller et al. (2014) found an association between lower fungal diversity and increased risk of asthma development later in life.

Despite the recognition of the importance for human health of exposure to bioaerosols, the precise role of biological agents in the development and exacerbation of symptoms and diseases is still only poorly understood. This relative lack of knowledge is mainly attributable to the lack of valid quantitative methods which could accurately measure fungal growth on materials or in the indoor air. Therefore, there is a clear need for improved microbial analysis methods that determine and measure reliably the presence and concentration of fungi and fungal particle if we are to better predict their health risks.

1.3 FUNGI AND FUNGAL GROWTH

Fungi are eukaryotic organisms that lack chlorophyll and obtain their nutrients from the growth media through the activities of their enzymes. On the other hand, moulds are filamentous fungi that grow with branched multi-cellular filamentous structures called mycelium (Eduard, 2006). In general, fungi are characterized by a visible vegetative body or a colony composed of a network of threadlike filaments which infiltrate into the

materials on which they feed. Fungi are usually saprophytic in nature; thus they obtain nutrients from dead organic matter provided that there is sufficient moisture. They can live off many of the materials present in the indoor environment such as wood, cellulose, insulations, wallpapers, glue and everyday dust and dirt (Adan, 1994; Foarde et al., 1996; Viitanen and Ojanen, 2007). Thus, fungi have the remarkable capability to degrade almost all natural and man-made materials (Hoang et al., 2010; Nevalainen and Seuri, 2005; Nielsen et al., 2004) especially if they are hygroscopic (Flannigan et al., 2002; Klamer et al., 2004). Fungi obtain nutrients by releasing extracellular enzymes and acids that break down the materials prior to their absorption. In the process, particles, including microbial degraded materials as well as gases, especially microbial volatile organic compounds (MVOCs), are released into the environment (Górny, 2004).

Most fungi have an outdoor origin and gain access to the indoor environment by infiltration and are carried inside by humans or pets (Adams et al., 2013; Amend et al., 2010; Pitkäranta et al., 2008; Pitkaranta et al., 2011). Some of the most abundant fungi measured in the indoor air and house dust, e.g., *Alternaria*, *Cladosporium*, *Penicillium*, yeasts, and *Aspergillus,* have been found in homes even in those not subjected to severe water damage (Chew et al., 2003; Horner et al., 2004; Meklin et al., 2004; Vesper et al., 2004). These fungal genera are common soil and leaf fungi (Horner et al., 2004). However, species like *Aspergillus versicolor*, and *Penicillium brevicompactum* are found at higher concentrations indoors than outdoors (Hyvärinen et al., 2001) indicating that they are actually generated indoors. Other taxa reflecting indoor fungal contamination include *Alternaria*, *Cladosporium, Penicillium, Epicoccum, Stemphylium, Phoma, etc.* (Adams et al., 2013). There are some fungal taxa commonly found on moisture-damaged materials indoors including *Aspergillus*, *Cladosporium*, *Paecilomyces*, and *Penicillium* (Andersen et al., 2011; Hyvärinen et al., 2002). Taxa such as *Aureobasidium* known to grow on moist surfaces in indoor environments have been

detected by next generation sequencing methods (Pitkäranta et al., 2008; Pitkaranta et al., 2011).

1.4 CONDITIONS THAT PROMOTE FUNGAL GROWTH INDOORS

1.4.1 Building characteristics

Distinct characteristics of the building can play an important role in the creation and accumulation of moisture which eventually damage the building materials as a consequence of mould growth on their surfaces (Odom and DuBose, 2000; Warscheid, 2011). In recent times, there have been attempts to minimize energy usage, and so buildings are designed with improved insulation and ventilation systems that meet the needs of maintaining thermal comfort. For example, a reduction in the ventilation rate is one way to achieve potential energy savings. On the other hand, continued running of the system at lower flow rates decreases the efficiency of the air exchange and this can allow moisture to accumulate, creating conditions favourable for microbes, especially fungi, to grow indoors (Lee et al., 2012).

In colder climates, buildings are constructed with very good insulation in order to reduce heat loss. In order to achieve good heat insulation properties, modern buildings are constructed using different materials in several layers (Burke et al., 2002). For example, the outer wall can be made of bricks, two types of boards, mineral wool and wood, plastic wraps and gypsum boards (Odom and DuBose, 2000). The materials serve as insulation to improve the thermal performance of the building envelope. Unfortunately, the building may become a microbiological reservoir and a contributor to the microbial exposure due to their ability to absorb and accumulate moisture (Kemp et al., 2003). Conventional and traditional houses, on the other hand, are built with fewer materials with a more homogeneous construction (Odom and DuBose, 2000), comprising of natural materials, in some cases, simply with bricks and wood.

Growth in population, upgrading of building services and comfort levels together with the increased time spent inside buildings have elevated building energy consumption (Pérez-Lombard et al., 2008). The increased energy consumption has led to increased CO_2 emissions from the built-up environments of developed countries. In turn, this has made strategies aimed at improving energy efficiency and saving overall consumption as a major target for energy policies in most countries (Annunziata et al., 2013; Pérez-Lombard et al. 2008). The need for a realistic solution to achieve the reduction of energy use in the building sector has triggered the call for near-zero-energy building in the European Union (EU). The European Commission has set a clear goal for energy efficiency, i.e., a 20% reduction in greenhouse gas emissions and an equivalent decline in energy consumption in new buildings by 2020. The European regulatory framework on energy performance of building directive 2002/91 EC mandates that all new buildings from 2021 will be near zero energy (Council, 2002). Over time, this would make these types of structures ubiquitous. As the term "near zero" suggests, these buildings would have very good insulation to make them as energy efficient as possible requiring little or no input from other sources (Annunziata et al., 2013; Desideri et al., 2013). However, it is not known how these new buildings will impact on the indoor microbiome and how in turn this will affect human health. Therefore, requirements and specification for new buildings are needed to maintain good indoor air quality.

Due to the heterogeneous nature of new buildings, there is a variety of materials that create micro-niches, i.e. they have a favourable temperature, water activity (a_w) and relative humidity (RH). For example, the surfaces of affected building materials (such as concrete and ceramic tiles in moist walls, ceiling tiles, dust particle laden wooden furniture) create specific niches suitable for the growth of microorganisms including bacteria and fungi. Not unexpectedly, the climate within the building varies from one part of the indoor environment to the next. Thus, fungal growth would also be predicted to vary with the microclimate

created. Moisture damage and dampness in buildings often affect a variety of structural components of building materials, leading to a deterioration of the indoor air quality.

1.4.2 Water, nutrients and temperature requirements

Water-damaged building materials, particularly those rich in organic matter, can support microbial growth if they remain wet for a prolonged period of time (Adan, 1994; Nielsen et al., 2004). When certain requirements are fulfilled such as temperature, nutrient and pH conditions, microbial growth can occur within an hour (McGinnis, 2007). Nonetheless, the principal limiting factor is the availability of moisture (Adan, 1994; Adan, 2011). It has been established that the lowest RH of a material at which fungi can grow is in a range around 75–80%, which corresponds to a water activity (a_w) of 0.75–80 (Adan, 1994; Grant, 1989; Rowan et al., 1999). The moisture of the substrate that is available to the fungi for growth is the so-called free water and this amount is influenced by the relative humidity of the surrounding air. This does not include bound water that is a component of the chemistry of the substrate (McGinnis, 2007). Moisture sources for fungal growth on materials indoors may be internal or external with moisture movement into and through building cavities by convection, gravity or capillary action.

Pasanen et al. (2000) have found that relative humidity values of 70–90% are required if there is to be fungal growth on building materials. Furthermore, the relative humidity required for growth depends on the particular material and the fungal species involved. Since most materials are porous in nature, adsorption of water into the materials first occurs via the pores before the material surface and become available to the microbes. Thus, porous materials support fungal growth when their RH is higher than 80% (Adan, 2011). These conditions influence the extent of colonization and the types of fungi that will be present, since any changes in moisture availability will change also the composition of the microbial species present in that environment. For example, certain species of *Penicillium*, *Erotium* and *Aspergillus* grow in

relatively dry environments with RH between 75–85% (e.g., in settled house dust on material surfaces with a relatively low RH). As RH increases, different species such as *Basidiomycetes* and *Eratonium* begin to grow, requiring continuously wet substrates such as soaked wallboard with RH range of 80–90%, while others like *Fusarium, Cladosporium* and *Stachybotrys* only grow at RH exceeding 90% (Dillon et al., 2005; Grant, 1989; Miller, 2011; Pasanen et al., 1992; Pasanen et al., 2000; Samson et al., 2010).

The above-mentioned conditions highlight that moisture management is critical for controlling fungal growth on building materials and this requires an understanding of the water-holding capacity (WHC) of the material, i.e., the maximum capacity of water that a material can hold when completely immersed in water, and/or the equilibrium moisture content (EMC), the total amount of moisture absorbed by the material and adsorbed by its surface. Both WHC and EMC are important indicators of risk for fungal growth on a material exposed to water (Hoang et al., 2010).

In addition to humidity and water, fungi need adequate nutrition and temperatures in order to grow. The availability of nutrients depends on the composition of the building material. Certain building materials like wood and ceiling tiles are organic in their nature; they contain complex polymers such as starch, cellulose and lignin. These components are broken down by the extracellular enzymes of the fungi into simple sugars, amino acids and other simple nutrients by fungi (D'souza et al., 1999; Sedlbauer, 2001). As fungi are able to utilize many complex polymers, a wide range of materials can act as nutrient sources. Fungal growth in moisture damaged materials eventually results in decay, discoloration and degradation (Murtoniemi et al., 2003).

Fungi can grow over a wide temperature range (5–39 ^{O}C), (Zak and Wildman, 2004). However, at low temperatures (0–5 ^{O}C), the fungal metabolic activities necessary for growth are slowed down, rendering the fungi dormant until an optimum temperature is

reached (Kubicek et al., 2007). At a higher temperature (34–36 ^{O}C) the metabolic reaction rates increase and at temperatures above 46 ^{O}C, the fungi become stressed and die (Nofal and Kumaran, 1999). This is because most of the activities of the fungi are dependent on DNA and enzymes.

1.4.3 Types of building materials

Fungal growth on building materials is dependent on the chemical composition of the materials (Hoang et al., 2010). The most susceptible materials to microbial growth and biodegradation are those with a natural organic composition e.g., wood and paper. These are materials containing starch, cellulose and hemicellulose, pectin and lignin in plants (D'Souza et al., 1999; Rantamaki et al., 2000; Viitanen et al., 2010). Based on these components, a wide variety of materials are potentially suitable for supporting fungal growth (Hoang et al., 2010; Nevalainen and Seuri, 2005; Nielsen et al., 2004).

Buildings contain a wide variety of materials that affect the germination and growth rate of fungi (Anagnost, 2007). Thus each material serves as a niche for a specific microorganism, depending on the composition of the material, water activity and nutrient content (Hoang et al., 2010; Pasanen et al., 2000). These properties of the building materials determine the diversity and extent of growth of the microbes (Andersen et al., 2011; Hyvärinen et al., 2002). Sedlbauer (2001) proposed four categories of building materials based on their water activity, composition and whether or not they are susceptible to microbial growth. Category 0 include building materials serving as an optimum culture medium; category I, biologically recyclable building materials; category II, building materials with a porous structure; and category III, building materials that are not degradable nor contain any nutrients. Based on these categories, the characteristics of each material will have a different effect on fungal growth.

Wood remains the most extensively used material in Finnish buildings (Hukka, 2000; Viitanen et al., 2010). Wood is able to absorb and retain water and moisture from both standing water and the environment (Viitanen, 2001; Viitanen et al., 2010). This characteristic in addition to the high nitrogen-bound compounds and low molecular carbohydrates that transferred to the wood surface during processing mean that wood is very susceptible to fungal growth (Viitanen, 2001). Certain species are commonly found on moisture-damaged wood e.g. *A. versicolor, P. brevicompactum*, (Andersen et al., 2011; Hyvärinen et al., 2002; Viitanen et al., 2010).

Gypsum board, on the other hand, is mostly used in the inner wall liners in buildings (Payne et al., 2000). The paper liners used to reinforce the gypsum core makes gypsum board susceptible to fungal growth. Since the inner core (gypsum) is able to retain water and make it available to the surface paper lining, there can be the prolonged presence of the water and moisture required to sustain fungal growth (Flannigan et al., 2002). While the inner core (gypsum) may not be susceptible to fungal growth, the glue and paper serve as good media due to their organic nature (Murtoniemi et al., 2003). The fungal species routinely found on gypsum board are the cellulolytic *S. chartarum* (Grant, 1989) and *C. cladosporioides* (Murtoniemi et al., 2003).

Plastic materials are also becoming more commonly used in buildings either as sheets or pipes. As sheets, they are used as material envelopes which insulate the building. Though plastics are known to be resistant to microbial attack because microbes do not possess any enzymes capable of degrading synthetic polymers (Mueller et al., 2013), the addition of plasticizers can make the plastics susceptible to microbial growth (Webb et al., 2000). These plasticizers are commonly organic acid esters such as dioctylphthalates (DOP) and dioctyladipate (DOA) which added to the polyvinyl chloride (PVC) to modify the polymer's physical or mechanical properties (Webb et al. 2000).

Glass fibres used in insulation materials do not support fungal growth. However, the glue used as binders does contain nutrients that may promote fungal growth (Payne et al., 2000) since these glues can be synthetic or plant-based. For example, the urea-based derivatives, polyurethanes, which are used as binders, are known to support fungal growth (Chang et al., 1995). Plant-based binders are commonly used in the new "green" acoustic boards (personal communication, Jyrki Kilpikari, Saint Gobain, Finland) and may also contain nutrients suitable to allow fungal growth.

Conventional building products often consist of a heterogeneous mixture of materials including adhesives and resins (e.g. urea–formaldehyde adhesives) that may pose health risks to the occupants of the building. Building materials labeled as "green" are becoming increasingly popular for in-home use and are frequently advertised as low- or no- chemical emission materials as well as being recyclable and less toxic (Spiegel and Meadows, 2010). There are different kinds of green materials including some sound-proofing ceiling materials fabricated from recycled cellulose, some gypsum boards that are reclaimed from used gypsum and recycled paper, and medium and high density fiber boards made from recycled wood and straw (Spiegel and Meadows, 2010). These "green" building materials are often rich in organic materials and can easily provide nutrients for microbial growth if sufficient moisture is available (Odom et al., 2008).

Although many conventional materials have been investigated to understand their susceptibility to fungal growth, there is a lack of information available on the susceptibility of "green" building materials to fungal and bacterial growth. Hoang et al. (2010) reported that cellulose-rich, organic materials present in "green" buildings are more susceptible to growth of *Aspergillus niger* than inorganic materials. It is therefore important to assess the microbiological safety of the new "green" and other building materials.

1.4.4 Contamination or soiling

All materials, both organic and inorganic, are able to sustain fungal life especially when the materials have dust, dirt or other deposits on their surface which represent sources of carbon and nitrogen (Foarde et al., 1996; Viitanen and Ojanen, 2007). Dust is known to contain microorganisms, debris and other animal or insect parts that serve as nutrients for fungal growth (Rintala et al., 2012). Thus, more growth is observed on materials with dust on their surfaces compared to those without dust (Foarde et al. 1996; Kowalski et al., 1999). Furthermore, settled dust or soil alters the water absorbing and retentive characteristics of the material surface, making the material surface continually moist, conditions in which fungi thrive (Flannigan et al., 2002). Dust absorbs water from the atmosphere. It has been shown that dust competes with the material surface for moisture, with the dust holding more water due to its more hygroscopic nature. Therefore, dust may promote fungal growth even on materials that naturally would not support microbial growth (Foarde et al., 1996; Viitanen and Ojanen, 2007). Thus, in indoor environments, it is important to select materials that are naturally resistant to dust accumulation if one wishes to minimize or eliminate the conditions that can lead to fungal growth (Hoang et al., 2010; Nevalainen and Seuri, 2005). Correspondingly, if one wishes to better model the real environmental conditions, the presence of dust on surfaces needs to be included in the research design when investigating the susceptibility of building materials to fungal growth.

1.5 AEROSOLIZATION OF FUNGAL SPORES AND FRAGMENTS

Fungal spores and hyphae usually originate from the colony surface by forces associated with turbulence, temperature, air velocity, vibration and zone of convection (McGinnis, 2007). Furthermore, the maturity of the colony, changes in temperature, relative humidity over the culture surface, light periods, nutritional composition of the substrate and the specific fungal

species will determine the frequency and the number of spores that will be liberated and transported into the air at any given time (McGinnis, 2007; Górny et al., 2001, 2001, 2003, 2004; Kildesø et al., 2003; Pasanen et al., 1991; Seo et al., 2008). The dispersal of fungal particles depends upon their size, shape, roughness, density, electrostatic charge, air movement and activities that influence the circulation of the air (McGinnis, 2007).

There are usually two primary mechanisms involved in the release of fungal particles; active and passive release (Adan, 2011). Active release refers to an adaptive type of particle aerosolization, via forces arising inside the fungi attributable to a burst of energy by a mechanism known as osmotic pressure and surface tension discharge (Bridge and Spooner, 2001). Passive release occurs by energy originating from outside the fungi, such as mechanical disturbances of the fungal colonies by mechanical handling, vibration or air currents. The latter forces can also cause secondary release of settled spores from surfaces. Activities that have been shown to increase fungal spore concentrations in indoor air include daily activities such as vacuuming, sweeping, walking etc. (Buttner and Stetzenbach, 1993; Chen and Hildemann, 2009; Lehtonen et al., 1993; Veillette et al., 2013).

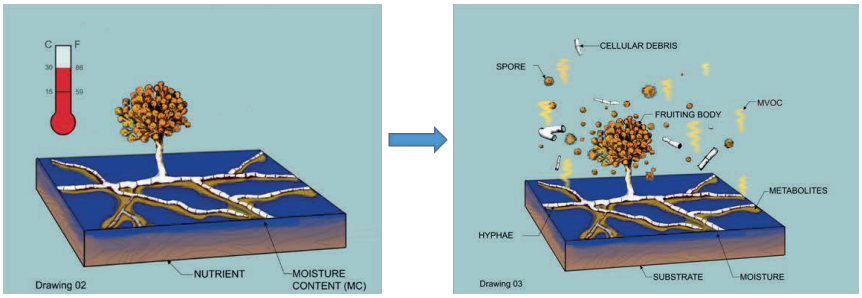

Fig. 1. Schematic diagram showing the growth of fungi on a material surface with the subsequent release of particles of the fungal growth (Morse and Acker, 2009). Reproduced with permission from The Whole Building Design Guide and The National Institute of Building Sciences.

During fungal growth and sporulation, as well as when the culture is in a dormant phase, there is the release into the indoor environment of spores and other sub-micrometer non-viable particles that contain bioactive agents (Afanou et al., 2015; Afanou et al., 2014; Cho et al., 2005; Górny et al., 2002; Górny, 2004; Madsen et al., 2005; Reponen et al., 2007). The small particles (d_a < 1μm) are referred to as fragments (Górny et al., 2002). They may be of biological origin derived from fragmented intracellular and extracellular structures of spores, conidia, hyphae, chlamydospores etc. (Fig. 1) (Afanou et al., 2015; Afanou et al., 2014; Cho et al., 2005; Górny et al., 2002; Górny, 2004; Seo et al., 2009). Hyphal fragments have been shown to represent 6–56% of the total fungal particles in field studies based on microscopic sample analysis (Foto et al., 2005; Green et al., 2005).

In addition, during metabolism, biodegraded growth materials (nutrient agar or building materials) (Brasel et al., 2005; Cho et al., 2005; Górny et al., 2002; Kildesø et al., 1999; Kildesø et al., 2003; Seo et al., 2009) are released. Furthermore, gases, for example, microbial volatile organic compounds (MVOCs) are also released (Górny, 2004; Warscheid and Braams, 2000). The MVOCs may form sub-micrometer particles through a process of secondary aerosol formation (Górny, 2004). These sub-micrometer particles have been shown to be aerosolized into the indoor environment following exposure to the effects of airflows and vibration (Górny et al., 2002; Kildesø et al., 1999; Madsen et al., 2005). Fragments have been found to constitute a large part of bioaerosols in terms of numbers (Górny et al., 2002; Green et al., 2005; Madsen et al., 2009; Tendal and Madsen, 2011), but no correlation has been found between the numbers of spores and fragments (Foto et al., 2005; Górny et al., 2002). The aerosolization of fungal particles in chamber studies have shown that fungal fragments are released at levels up to 514 times higher than spores (Cho et al., 2005; Górny et al., 2002; Kildesø et al., 2003; Madsen et al., 2005; Reponen et al., 2007). On the other hand, in an earlier study, Li and Kendrick (1995) used microscopic counting and found that hyphal fragments accounted for only 6.3% of the total number of

fungal particles in indoor environments in Canada. In addition, by applying a biomass determination, Adhikari et al. (2012) detected lower amounts of NAHA enzyme in fungal fragments < 1 μm compared to spores > 1.8 μm.

Though both types of particles (spores and fragments) released from the fungal cultures during aerosolization are potentially harmful, the fragments are of greater importance since they tend to remain suspended longer in air than the spores (Górny et al., 2002; Górny, 2004; Kanaani et al., 2008; Madsen et al., 2005; Reponen et al., 2007) and have a tendency to penetrate deep into alveolar regions of the respiratory tract when inhaled (Cho et al., 2005; Miller et al., 2003). Cho et al. (2005) have used a computer-based model to assess the deposition of spores and fragments of *A. versicolor* and *S. chartarum* in the respiratory tract. For both fungi, they found that the vast majority, 65 – 90%, of inhaled fungal spores deposited in the nasal and extrathoracic regions while only 3 – 15% and 2 – 5% of the spores deposited in the alveoli-interstitial and bronchial-bronchiolar regions respectively. They also demonstrated that about 60% of fungal fragments deposited in the alveoli-interstitial region with 14 – 15% being trapped in the nasal and extrathoracic regions. It can therefore be deduced from the above modeling analysis that the different deposition efficiencies could have consequences on the potential adverse health effects induced by inhaled fungal particles of different sizes.

Fungal fragments have been shown to contain antigens (Górny et al., 2002; Górny, 2004), allergens (Green et al., 2006; Green et al., 2009; Green et al., 2005), mycotoxins (Brasel et al., 2005; Seo et al., 2008; Sorenson et al., 1987), and (1→3)-β-D-glucans (Reponen et al., 2007; Seo et al., 2008). Their size in relation to their numbers and their biological properties all contribute to their potential to evoke adverse health effects. It is known from atmospheric studies investigating the adverse health effects of ultrafine particles that it is number concentration rather than mass

concentration which is important (Penttinen et al., 2001; von Klot et al., 2002).

Indoor air, like outdoor air, has many sources of contaminants that affect health adversely. However, it is not clear which source is associated with the adverse health effects. Therefore in indoor environments, it is important to characterize fungal fragments based on their origin since this knowledge can improve our understanding of the potential adverse health effects associated with exposure to these particles.

Different fungal species have characteristic structures and thus behave differently when they become airborne. In addition, the growth substrate which is providing the nutrients for the fungi may also affect the properties of the spores and fragments and could contribute to fragments released from the biodegradation of the substrate itself during fungal metabolism. The amount of fungal particles released may also depend on the type of substrate and the conditions under which the fungi were grown. It is very important to evaluate spore properties under a variety of conditions in order to gain insights into the contribution of these factors to the properties of the spores.

1.6 ANALYTICAL METHODS FOR MEASURING FUNGAL CONCENTRATIONS

Measurements of fungal concentration in indoor air and on materials are very important in determining the sources and nature of fungal contamination which in turn helps in estimating the risks associated with exposures to fungal particles in moisture damaged buildings. Since fungi grow differently on different materials in a building and are affected by the diverse and changing conditions in the indoor environment, methods that accurately estimate the amounts of fungi are required. Quantitative as well as qualitative methods are usually employed.

The quantitative measurements assess how many fungi are present on material surfaces or in the air being determined by techniques such as the culture-based methods, microscopic counts and molecular methods. These methods determine the extent of growth, sporulation and total number of cells (Krause et al., 2003). Total fungal biomass has been used as a surrogate of the overall fungal exposure. Total fungal biomass can be determined using chemical markers that are found in the fungi. The chemical markers include ergosterol (Szponar et al., 2003), N-acetylhexosaminidase (NAHA), (Reeslev et al., 2003; Rylander et al., 2010), and 1→3-β- glucan (Foto et al., 2005). Each one of the methods is thought to provide a different perspective of fungal quantities since they evaluate specific responses of the various stages of fungal growth. However, no previous study has comprehensively compared several assay methods side-by-side. Simultaneous comparison is valuable if one wishes to evaluate the pros and cons of the different assay methods in quantifying the fungal concentration and in determining how fungal concentrations are reflected in the methods.

1.6.1 Cultivation method of determining fungal growth

Traditional quantification of fungi is based on the determination of the number of colony forming units (CFU). This method is useful in identifying microbes up to the genus level (Burges and Otten, 1999) since it allows the qualitative and quantitative assessment of an investigated environment (Ettenauer et al., 2014). Thus, it gives useful information which can help to confirm an environmental source for an outbreak investigation. Counting culturable microorganisms is potentially a very sensitive technique, allowing the identification of many different fungi in a sample. This method also allows for a qualitative assessment of exposure by identifying the genus of fungi since not all fungi pose the same hazard. After cultivation, fungi can be identified to the species level using Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF-MS) (De Carolis et al., 2012).

However, the culture method has its inherent limitations, such as the inability of a particular media to satisfy the specific growth requirements of all fungal species (Douwes et al., 2003). The choice of growth media used can contribute to substantial variability of the types and amounts of species that are cultured. Cultivation method lacks the ability to detect non-culturable and dead microorganisms, cell debris and microbial components, although they too may have toxic or allergenic properties (Green et al., 2005). It has been demonstrated that culturability of indoor fungi varies widely (< 1–100%) for all known fungal species grown by cultivation (Lee et al., 2006; Meklin et al., 2004; Toivola et al., 2002). Consequently, the total quantity and diversity of fungal cells are usually drastically underestimated by culture-based methods (Bridge and Spooner, 2001; Douwes et al., 2003). In addition to these limitations, the cultivation method is very laborious and requires a long incubation time (minimum of 1 week) to detect fungal growth (Douwes et al., 2003). Since culture-based methods measure only a fraction of sampled fungi, CFU counted cannot completely characterize the fungi that might influence human health and well-being.

1.6.2 Microscopic spore counting

To circumvent the problems associated with culture-based detection techniques, methods that detect both viable and non-viable cells using microscopic counting have been developed (Bauer et al., 2008; Ho et al., 2005; Palmgren, 1986; Sattler et al., 2001). Simple light microscopy may be used to count microorganisms, but counting is based only on morphological recognition, which may result in severe measurement errors (Douwes et al., 2003). In epifluorescence microscopy, dyes such as acridine orange which stain the spores' DNA, are used to help in counting spores. The dye makes the spore fluoresce when viewed under the microscope (Palmgren, 1986; Thorne et al., 1994). The microscopic spore count method has its own limitations which include the masking of spores by large particles and the inability of some spores to absorb the dye (Burge, 1995). In addition, the spore counting approach is time consuming. The

method is, however, relatively cheap to perform and gives a general indication of atypical indoor fungal growth (Douwes et al., 2003).

Electron microscopy (EM) or scanning EM can also be used and it provides a better determination of spore counts and concentrations (Eduard et al., 1988; Karlsson and Malmberg, 1989). However, the different sources and similarities in particle appearance with particles from other sources may lead to difficulties in their quantification (Wittmaack et al., 2005). A good technique developed to circumvent this problem is the use of SEM microscopy coupled with energy dispersive X-ray spectroscopy (EDX). This analysis is based on determining the elemental composition of the particles after they have been identified with SEM. The elemental composition of biological particles differs from other particles and they also behave differently from non-biological particles. Based on this property, Matthias-Maser and Jaenicke (1991, 1994) developed criteria for determining primary biogenic organic aerosols (PBOA) in atmospheric samples. These were based on the detection of minor amounts of K, P, S, Na and Ca (usually <10% of relative element of X-ray intensity of the particle). This criterion was recently adopted (Coz et al., 2010) to characterize PBOA in the atmosphere. There are however, no previous studies which have characterized the origin of PBOA from fungal contaminated building materials in indoor environments based on their elemental composition.

1.6.3 DNA-Based methods

The DNA-based method, similar to total spore counting approach, detects both culturable and unculturable spores. DNA-based methods in addition, measure mycelial cells (Gonzalez and Saiz-Jimenez, 2004; Herrera et al., 2009; Meklin et al., 2004; Yamamoto et al. 2010). The molecular methods most often used in fungal studies include conventional or quantitative PCR (qPCR) specific for fungal species or groups (Haugland et al., 2004; Zeng et al., 2006), universal fungal PCR combined with denaturing gradient gel electrophoresis (DGGE) or temperature gradient gel

electrophoresis (Gonzalez and Saiz-Jimenez, 2004) terminal or conventional restriction fragment length polymorphism analysis (Buttner et al., 2007). Other methods include the molecular tracer methods (Elbert et al., 2007) and ribosomal DNA amplicon sequencing or metagenome analysis (Frohlich-Nowoisky et al., 2009; Liu et al., 2012; Pitkäranta et al., 2008; Tringe et al., 2008).

The advantages of using the DNA-based methods for quantifying fungal amounts instead of cultivation-based methods and microscopic counts are the speed, accuracy, and analytical sensitivity of this approach and the possibility to detect and identify dead or dormant microorganisms (Ettenauer et al., 2014). The ability of DNA-based methods to detect dead or dormant cells is important in indoor studies since the main exposure hazards relating to indoor microbial contamination are not dependent on viability. Quantitative PCR has provided valuable information on the occurrence of the most common indoor fungi, and this method shows great promise in being able to provide fast quantitative data on the occurrence of the studied organisms (Meklin et al., 2004; Pietarinen et al., 2008; Pitkaranta et al., 2011). Primers and probes are generally designed for the detection of a given genus (genus-specific primers) or for the detection of a single species (species-specific primers). The 18S or 16S ribosomal RNA genes can be used in the design of genus-specific primers and probes because they contain sequences that are highly conserved between members of the same genus, but the sequences are variable among different genera (Haugland et al., 2004).

There are a few publications describing the exploitation of qPCR analytical methods in studying the concentrations of species in indoor dust and air samples (Haugland et al., 2004; Kaarakainen et al., 2009; Meklin et al., 2004; Vesper, 2007) and building materials (Pietarinen et al., 2008; Pitkäranta et al., 2011). Individual fungal species have also been examined. For example, real-time PCR methods have been utilized to detect and quantify *Cladosporium* (Zeng et al., 2006) and *Aspergillus* (Goebes et al.,

2007) at the species level. Similar methods have been developed for measuring species of common indoor fungi such as *Aspergillus, Cladosporium, Penicillium* and *Alternaria* (Haugland et al., 2004; Meklin et al., 2007; Vesper et al., 2005). PCR methods make it possible to assess the presence and amounts of large groups of microorganisms. For instance, a quantitative PCR method has been used to measure 36 indicator species commonly associated with damp houses in the United States which has then been used to define an "environmental relative mouldiness index" (ERMI) for houses in that country (Vesper, 2007).

A recent approach for characterizing fungal populations is to apply next generation sequencing (NGS) methods that deliver fast, inexpensive and accurate genome information (Metzker, 2010). These methods can be used to identify a rare transcript without prior knowledge of a particular gene and can provide information regarding alternative splicing and sequence variations in the identified genes. By using NGS methods, the very wide diversity of indoor fungal flora has been revealed in house dust (Amend et al., 2010; Pitkäranta et al., 2008). In addition, the diversity and distribution of fungi on indoor surfaces sampled from non-moisture damaged homes (Amend et al., 2010) and both damaged and non-damaged homes have been studied via pyrosequencing (Kettleson et al., 2015). Some sequencing studies have only identified fungi to the genus level (Adams et al., 2013: Hoisington et al., 2014). However, sequencing of the internal transcribed spacer (ITS) region of the fungal ribosomal DNA makes possible identification down to the species level (Schoch et al., 2012). The sequencing of the ITS region provides qualitative information about the diversity of a fungal community; information that neither culture-based nor qPCR methods can provide (Dannemiller et al., 2014).

1.6.4 Chemical methods

Chemical methods assay cellular constituents, usually microbial cell wall agents (MCWA), metabolites of microorganisms or enzyme activities as surrogate measures microbial exposure,

instead of counting culturable or non-culturable microbial particles. Markers for the assessment of fungal biomass include ergosterol, (Miller et al., 1988; Szponar et al., 2003), fungal extracellular polysaccharides (ESP) and 1-3-β-glucan (Douwes et al., 2000; Sonesson et al., 1988). ESP even allows a partial identification of the fungal genera present. Microbial volatile organic compounds (MVOCs) produced by fungi may be suitable markers of fungal growth (Dillon et al., 2007; Moularat et al., 2008). The levels of toxins (e.g., mycotoxins) or pro-inflammatory components (e.g., bacterial endotoxins), non-toxic molecules (e.g., β-glucans) as well as the activity of N-acetylhexosaminidase enzyme can be used as chemical markers of large groups of microorganisms or specific microbial genera or species.

There are several advantages associated with these assay methods i.e. the stability of most of the measured components, allowing longer sampling times for airborne measurements. Standards can be used in most of the methods and there is usually the possibility of testing for reproducibility. Furthermore, the samples can be stored by freezing before use. However, these methods do not leave fungal isolates for further investigation once used.

Ergosterol

Ergosterol is a sterol present within fungal cell membranes and is considered an indicator for total fungal biomass (Szponar et al., 2003) in dust (Saraf et al., 1997), in building materials (Gutarowska and Piotrowska 2007; Szponar et al., 2003), and indoor air (Park and Cox-Ganser 2011). Ergosterol contents are measured by gas chromatography-mass spectrometry (Miller et al., 1988; Szponar et al., 2003). The presence of ergosterol in fungi has made this constituent a useful biomarker of fungal biomass in environmental assessments (Mille-Lindblom et al., 2004). On the other hand, it has been shown that ergosterol is labile and thus its concentration declines after the death of fungal hyphae (Gutarowska and Piotrowska 2007; Mille-Lindblom et al., 2004). However, it is not known how long the ergosterol stays stable in

the fungi. Ergosterol determination in samples is not done routinely, is generally rather expensive and requires a large amount of infrastructure and time to process and analyze samples.

Beta-glucan

Beta-glucans are polysaccharides found in the outer cell membrane of fungi, higher plants and some bacteria. In the fungal cell wall, glucans comprise a three-dimensional network of (1→3) and (1→6)-β D-linked anhydroglucose repeat units that are connected to other carbohydrates, proteins and lipids. (1→3)-β-D-glucan polymers can exist as a stable complex of three polymer strands forming a triple helix. The triple helical structure is generally considered to be the preferred form in nature (Young and Castranova 2005). From a quantitative point of view, (1→3)-β-D-glucans are the main constituent, accounting for between 47 to 60% by weight of the cell wall (Young and Castranova 2005). Therefore, it would be expected that the indoor species with the highest prevalence should contribute most to the (1→3)-β-D-glucan content. Measurements of (1→3)-β-D-glucan in indoor air have been done with a glucan-sensitive preparation of the Limulus amebocyte lysate (LAL) assay (Iossifova et al., 2009; Iossifova et al., 2007). Other methods for the analysis of fungal glucans have been based on antibodies. For example, Douwes et al. (1996, 1998) developed an inhibition enzyme immunoassay (EIA), which is specific to the (1→3)-glycosidic linkage and to water-insoluble glucans but less sensitive than the LAL test.

NAHA enzyme activity

The enzyme β-N-acetylhexosaminidase (NAHA) activity is one way of measuring fungal total biomass (Reeslev et al., 2003; Rylander et al., 2010). This method is based on a fluorescence labeled substrate which can be cleaved by the enzyme found in fungi. The amount of fluorescence detected is proportional to the amount of biomass present (Reeslev et al., 2003). By using enzyme activity as an indicator for fungal biomass, a fluorometric detection method can be used to determine whether fungal

growth is present on a building material. Furthermore, the extent of mould-affected materials and the efficacy of cleaning after remediation efforts can be monitored since the method is fast and can be done onsite. Beta-*N*-acetylhexosaminidase activity was shown to correlate with the fungal index molecules ergosterol and the phospholipid fatty acid 18:2ω6 in soil samples (Miller et al., 1988). Significant correlations have been reported between NAHA and total spore counts in dust (Madsen, 2003; Madsen et al., 2009), fungal biomass (gravimetric weight) and NAHA in fungal species grown on nutrient agar and between ergosterol content and NAHA activity on fungal contaminated gypsum boards (Reeslev et al., 2003).

NAHA is present in both the growth and stationary phases of fungal growth (Rast et al., 2003; Reeslev et al., 2003). Rylander (2015) reported that NAHA activity is retained during the first month of storage but slightly declines after a year. Compared to detection of ergosterol, NAHA enzyme activity measurements are much simpler to perform and can even be conducted on-site.

1.7 REAL-TIME DETECTION OF AIRBORNE FUNGAL PARTICLES

It has been stated that a thorough understanding of the significance of microbial exposure in indoor environment is lacking due to methodological difficulties in identifying and enumerating various microbial components (Green et al., 2006). Traditional bioaerosol detection methods such as the Andersen impactor and filter sampling require a separate step for analysis before concentration can be determined and result in relatively low time resolution (Górny et al., 2002; Reponen et al., 2011). These methods are well-established for culture-based and microscopic analyses which usually help in the identification of different fungi at the genus level. Sample collection usually only lasts for a short period of time. Thus, samples collected by these methods provide information on the concentration of the organism only at the time of sampling (Reponen et al., 2007).

Consequently, it is important to have measurement tools that permit sampling and analysis in real-time and with high resolution. Real time measurements would allow understanding of emissions and the temporal variation of airborne concentrations. Therefore, real time detection techniques are needed in various fields, e.g., bioprocess monitoring (Ganzlin et al., 2007), health related applications (Elston, 2001), environmental, defense and public security (Davitt et al., 2005; Kanaani et al., 2007; Méjean et al., 2004; Sivaprakasam et al., 2004).

Direct reading instruments that measure particles in real-time have been used in several laboratory studies, e.g. optical particle counters (OPCs) which measure particle concentration in the size range of 0.3–20 μm based on their light scattering properties. Applications of the OPC in the laboratory include the measurement of fungal spore concentrations after their release from fungal contaminated surfaces (Górny et al., 2003; Górny et al., 2002; Seo et al., 2007) and testing the performance of Air-O-Cell, Burkard and Button Samplers for total enumeration of airborne spores (Aizenberg et al., 2000). There are other direct reading instruments; the electrical low pressure impactor (ELPI), a multistage impactor, which has been used in evaluating the performance of N95 respirators (Lee et al., 2006) as well as to determine the aerodynamic characteristics of fungal fragments (Cho et al., 2005). The ELPI classifies aerosol samples into size fractions over a size range of 0.07–10 μm (Marjamäki et al., 2000). Prior to the impactor stages, the aerosols are charged in a corona charger and the charge evoked by the impacted particle is measured as a current by electrometer connected to each impactor. The aerodynamic particle sizer (APS), measures the dynamic size distribution of particles in the size range of 0.5–20 μm by determining the time-of-flight of individual particles in an accelerating flow field (Bartley et al., 2000). The APS has been used to measure the aerodynamic diameter of spores aerosolized from wet building materials (Kildeso et al., 2003). Particle concentrations in the size range of 0.02–1 μm can be measured using the P-Trak. This instrument measures the number

concentration of particles by saturating them with either water or alcohol vapour and cooling them so that their enlarged particle sizes can be detected by optical methods. Górny et al. (2002) and Seo et al. (2007) have used the P-Trak to measure sub-micrometer fungal particles in the laboratory.

Although very useful in laboratory-based studies where other particles can be eliminated, especially particles within a certain size range, the above described instruments have limited utility in the assessment of bioaerosol exposures because they are not specific since the process of distinguishing microbial and non-microbial particulate matter is complex (Green et al., 2011).

The Laser Induced Fluorescence (LIF) technique enables real-time detection of biological aerosol particles. LIF techniques have been developed to detect biological warfare agents (BWA) (Hairston et al., 1997). The LIF-instruments have not yet been widely applied in other fields of bioaerosol detection, partly because they have been optimized for the military use. More recently, LIF spectra were used to study pure cultures of fungal strains both in the laboratory (Bengtsson et al., 2005; Rativa et al., 2007) and in remote sensing applications (Raimondi et al., 2007). The use of LIF techniques can give insight into the origin of the fluorescent spectral features and contribute to the interpretation of data obtained using other fluorescence-based techniques.

The best known and most widely used LIF device is the Ultra Violet aerodynamic particle sizer (UVAPS). Other devices include the BioScout and Wide Issue Bioaerosol Sensor (WIBS-3) and the waveband integrated bioaerosol sensor (WIBS-4). The basis by which all of these devices detect depends on the fluorescence of compounds such as reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H), flavins, melanin, carotenoids, phenols, terpenoids, and DNA (Després et al., 2007; Frohlich-Nowoisky et al. 2009; O'Connor et al., 2011; Pöhlker et al., 2012; Raimondi et al., 2009; Saari et al., 2013) present in all living cells but at different proportions and measured at selective

wavelengths. Thus, LIF enables the differentiation of bioaerosols from other particles because of their fluorescence capabilities (Hill et al. 2013; Pöhlker et al. 2012). These devices have been used in measuring biological particle concentration in pig enclosures (Agranovski et al., 2004), outdoor environments (Huffman et al. 2010; Huffman et al., 2012) and classrooms (Bhangar et al., 2014). The UVAPS measures both aerodynamic particle size and autofluorescence of a single particle. The WIBS, on the other hand, measures optical size and the autofluorescence of bioaerosol particles (Gabey et al., 2010; Healy et al., 2014) by utilizing two excitation wavelengths and detecting two bands of fluorescence. The BioScout measures both autofluorescence and optical particle size of single particles using continuous wave laser diode and light scattering. Saari et al. (2014) have used both BioScout and UVAPS to measure fungal spores in the laboratory and found the former device to be more sensitive. This indicates that the LIF devices may have varying capabilities in detecting biological particles based on the wavelengths of light used and the type and amount of fluorescent compounds present in the samples. Data obtained from the use of these devices have usually been on fungal spores and not on small-sized fragments. This is because very little or no fluorescence is given off from fungal fragments compared to larger spores (Kanaani et al., 2008; Saari et al., 2014).

Different fungal species have characteristic structures and therefore, may have different biochemical compositions which in turn could influence the autofluorescence. Therefore, a variety of factors may affect the fluorescent properties of fungi under various conditions. These factors include fungal species, growth substrate, air velocity and age of the culture. The effects of these factors on the fluorescent properties of fungal spores measured with different LIF devices side-by-side would help with instrument calibration and ease the interpretation of LIF-based field results. This is because in a building, different materials serve as substrates for fungal growth. Furthermore, the fugal growths may be at different ages and be experiencing varying air velocities over their surfaces.

1.8 RATIONALE OF THE STUDY

The current study was carried out with the following motivation:

Most studies investigating the susceptibility of building materials to microbial growth have focused on the traditional materials. Though so-called green building materials have been available for some time, little is known about their ability to support microbial growth and hence their contribution to indoor air quality. Therefore, there is a need to compare both green and non-green building materials to evaluate their susceptibility to microbial growth.

There is no simple method which can estimate fungal growth at different time points. To date, comparisons between fungal quantification methods have rarely taken into account the temporal variation in the amount of fungi measured. The use of different methods may determine growth variations due to temporal effects.

Different fungal species have different structures, and thus their fluorescent biochemical composition may be distinctive. Furthermore, fungi grow on materials with varying nutritional contents and they are exposed to air currents of different velocities. All these factors affect the properties of fungal spores released from the growth. Information is needed to clarify the major factors that can affect the fluorescence properties of airborne fungal spores using LIF devices.

Fragments aerosolized from building materials could be a potential health hazard, depending on their origin and composition. Although some studies have used chemical, immunological and microscopic methods to characterize fragments, as far as is known, none of them was able to differentiate biological from non-biological fragments. Determining the origin of fragments will improve our

understanding of the potential health risks associated with exposures to fragments.

1.9 AIMS OF THE STUDY

The aims of this work was to study the microbial contamination of building materials by evaluating how fungi grow on different building materials and to examine their properties when they are aerosolized from the materials surfaces in laboratory settings. More specifically, the aims were:

> **Aim 1:** To experimentally evaluate the susceptibility of building materials (green versus non green) to microbial growth.

This was achieved by incubating three common indoor fungi (*A. versicolor, C. cladosporioides* and *P. brevicompactum*) on green and non-green building materials at a relative humidity of 95 – 97% for 4 weeks. Fungal growth on the two types of materials was evaluated using the NAHA enzyme activity and the cultivation method. This aim was achieved in the experiments conducted in paper I.

> **Aim 2:** To apply emerging analytical methods – NAHA enzyme and qPCR based analysis – to measure microbial biomass as an index of microbial growth.

Five different fungal growth assay methods (cultivation, total spore count, qPCR, ergosterol content and NAHA enzyme activity) were used to determine fungal growth. The methods were compared in terms of their ability to determine temporal variation in the amounts of fungi measured. Paper II addresses this aim.

> **Aim 3:** To apply LIF techniques in determining the effects of various factors on the fluorescent properties of fungal spores released from fungal contaminated material surfaces.

Two LIF devices (UVAPS and BioScout) were used to measure the fluorescent properties of fungal spores released under various conditions. The effects of fungal species, cultivation time, growth substrate, and air velocity on the fluorescent properties of released spores were evaluated. Paper III addresses this aim.

> **Aim 4:** To aerosolize and characterize fungal fragments from contaminated surfaces.

Fragments released from fungal growth were characterized using SEM-EDX analysis in order to distinguish biological fragments from non-biological counterparts. Indicator elements (N and P) detected in fungal spores and growth but not in the blank growth materials were used to differentiate biological fragments from non-biological ones. This aim was achieved in Paper IV.

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6 General discussion

6.1 MEASUREMENT OF FUNGAL GROWTH ON DIFFERENT BUILDING MATERIALS

The classification of building materials into either green or conventional materials did not significantly affect the growth of the fungal species examined in this thesis. This finding is in agreement with another report (Hoang et al., 2010); these workers also found no significant difference in growth of fungi on the two classes of building materials. In this study, the extent of growth on the two classes of building materials was, however, found to depend on the chemical composition, nutritional value and moisture availability (I). Dust was used to simulate realistic indoor conditions in construction niches and it served as an external source of nutrients. In the presence of dust, the extent of fungal growth was elevated on all the materials, irrespective of their chemical composition or nutritional value. Other studies that have included dust on material surfaces such as insulation materials, fiberglass, wood and steel also observed increase in fungal growth (Foarde et al., 1996; Pasanen, 1998; Viitanen, 2001). In the study of Hoang et al. (2010), external nutrient sources with and without carbon were used. They observed considerably less fungal growth when spores were inoculated with yeast nitrogen base which contained all required nutritive components and vitamins except carbon. They therefore speculated that fungal growth on certain building materials may be carbon-limited in the short term but not permanently. Dust is known to contain debris of various materials including parts of dead plants, insects, animal debris which are rich sources of carbon and nitrogen (Rintala et al., 2012). Thus, in the presence of dust, almost all materials may be susceptible to fungal contamination. Therefore, to minimize fungal growth on the surfaces of different materials, dust generation and accumulation should be minimized.

The effects of the chemical composition and nutritional value of the materials themselves were assessed by using material samples inoculated without dust. It was found that wood and gypsum boards without dust had more fungal growth than green acoustic board (ABD), conventional acoustic board (ABY) and Haltex (porous wood fiber board) (I). Wood contains a relatively high nutrient content (Viitanen and Ojanen, 2007) and therefore it is very sensitive to fungal growth. In addition, the high cellulose and starch contents of wood makes it capable of supporting fungal growth at the lowest water activity (Hoang et al., 2010; Nielsen et al., 2004; Viitanen et al., 2010). Gypsum board, on the other hand, has paper glued with starch on its surface. Both the paper and glue are rich sources of nutrients for fungal growth. The gypsum core is able to retain water and thus it can supply the paper surface with enough moisture even at lowest moisture content (Miller, 2003).

The two acoustic boards, however, have glass fibers which have low nutrient contents. Inorganic materials are known to require higher moisture contents to allow fungal growth compared to their organic counterparts (Pasanen, 2000; Viitanen et al., 2000). In the present study, wood, ABD, ABY and Haltex had higher moisture and normalized moisture contents than gypsum board, however, increased fungal growth was observed on wood and gypsum board compared to the acoustic boards. Thus, fungal growth on building material surfaces in indoor environments can be reduced if one can eliminate moisture accumulation in the building materials, especially organic based materials.

No previous studies have comprehensively compared several assay methods side-by-side. Study II compared simultaneously five different methods in quantifying fungal concentrations. Previous studies have compared the cultivation method with either qPCR techniques (Lignell et al., 2008; Meklin et al., 2004; Pietarinen et al., 2008), ergosterol assay (Pasanen et al., 1999), NAHA assay (Reeslev et al., 2003) or total spore count (Eduard, 2003). The simultaneous side-by-side comparison of the five

assay methods used in this study revealed differences in their sensitivity at detecting the temporal changes in fungal concentration. Varying growth dynamics were also observed for the different species over time. The differences in results obtained by the methods were mainly dependent on the physiological state of the cells, especially the balance between growth and death. Each of the assay methods provided a different perspective of fungal quantification due to their specific responses to various stages of fungal growth.

In terms of concentrations, qPCR and total spore count estimated the highest cell concentrations, whereas cultivation method gave the lowest value (II). This is in agreement with other studies (Lee et al., 2006; Meklin et al., 2004; Pietarinen et al., 2008). In addition, the cultivation method was found to lack reproducibility, and therefore was not rated as a reliable method for repeated measurements (II). However, the cultivation method proved to be the most sensitive for determining temporal variation on growth as well as transient changes in growth dynamics. The cultivation method measures culturable cells (Meklin et al., 2004), and therefore, the effect of material and other factors that influence growth dynamics will impact on its outcome. Non-culture methods, on the other hand, measure both culturable and non-culturable cells (Amann et al., 1995; Niemeier et al., 2006) and thus they fail to reveal the dynamics of the fungal growth. However, they are useful when estimating the total fungal biomass. For example, ergosterol and NAHA enzyme activity have been detected in both fungal spores, both dead and alive, and fragments (Pasanen et al., 1999; Reeslev et al., 2003; Rylander et al., 2010; Szponar et al., 2003). Therefore, their measurements can be used as surrogates for total fungal biomass and help in determining total fungal exposures. Despite the differences in measurements of the physiological states of the fungal growth, a moderate to good correlation between the assay methods was observed. This indicates that the methods reveal similar trends for fungal growth.

6.2 AEROSOLIZATION AND CHARACTERIZATION OF FUNGAL SPORES AND FRAGMENTS

The fluorescent properties of fungal spores released from fungal contaminated materials were affected by the growth substrate, fungal species, age of the culture and air velocity to which the cultures had been exposed (III). The same factors affected the fragment/spore ratios (IV).

Spores obtained from cultures on gypsum board had lower fluorescent properties than spores from agar indicating that cultures growing on nutrient poor substrates contain less compounds capable of fluorescence. Previous studies with fluorescence measuring devices in laboratory settings have grown the cultures on agar media which have optimum nutrients (Agranovski et al., 2004; Kanaani et al., 2007). This invariably resulted in good detection efficiency of the instruments. The low detection of fluorescence in spores aerosolized from gypsum board indicates that spore concentrations may be underestimated when the fluorescence-based devices are used in field studies, where fungal spores may originate from building materials poor in nutrients. The underestimation of spore concentration by the UVAPS was observed when OPC was used in parallel to measure spore concentrations.

From the results of this thesis, no significant differences in spore concentrations were observed when the spores were aerosolized from wood, gypsum board and agar (IV). On the hand, the fragment/spore ratio (F/S) for agar was higher compared to wood and gypsum board. These results are in agreement with those of (Seo et al., 2009) who observed a higher F/S ratio for *A. versicolor* cultivated on MEA than on gypsum board and ceiling tiles. Generally, higher concentrations of fungal particles are aerosolized from dry surfaces with low moisture contents than wet surfaces with high humidity (Górny, 2004). Agar may have a different moisture content and moisture dynamics during the fungal than wood and gypsum. During growth, the moisture

content becomes reduced (Seo et al., 2008) and it is possible that agar loses more moisture than wood and gypsum. For this reason, fungal growth on agar undergoes desiccation stress and releases more fragment particles than growth on wood and gypsum board.

The effect of species was seen in the lower fluorescent particle fraction (FPF) values for *C. cladosporioides* compared to *A. versicolor* and *P. brevicompactum*. This finding is consistent with that observed by (Healy et al., 2014) who used UVAPS and WIBS-4. They attributed the insensitivity of the real-time LIF devices to detect *C. cladosporioides* to the dark-skinned nature of these spores, preventing impinging photons from penetrating to reach the exterior pigments to excite fluorescence from internal fluorescence. This indicates that *C. cladosporioides* concentrations may be underestimated in field measurements. The present results also showed that *A. versicolor* produced a higher F/S-ratio compared to *C. cladosporioides* and *P. brevicompactum.* The results are consistent with the findings from Afanou et al. (2014). They attributed the increased sub-micrometer fragments from *A. versicolor* to the outer-wall spines which are easily sheared away during sampling.

Fragment/spore ratio (F/S) had an increasing trend with increasing age of the culture although this was not statistically significant (IV). In this study, moisture content of wood and gypsum increased from day 0 till the time point of the aerosolization experiments. Furthermore, prior to the aerosolization, all the material samples were dried overnight to allow better release of fungal particles. With differences in the absorption and retention of moisture by the various materials, fungal biomass was also affected and hence affected the release dynamics of fungal particles from the material surfaces. Seo et al. (2009) demonstrated that F/S increased with age, a phenomenon they attributed the increase in particle release from older cultures to changes in fungal biomass and moisture content (Seo et al., 2008). Dryness on the surface of the culture increases the

aerosolization of fungal particles by reducing the adhesion forces between the fungal structures and making these structures more brittle (Seo et al., 2009). Therefore, it has been concluded that with time, fungal growth in buildings may increase the contribution of sub-micrometer-sized fungal fragments to the overall mould exposure (Seo et al., 2009). Spores aerosolized from older cultures displayed lower fluorescence than younger cultures (III). This finding is in agreement with the findings obtained by Kanaani et al. (2007) who reported a decrease in fluorescence emitted by *Penicillium* and *Aspergillus* from 2 days to 21 days. They suggested that fluorescent intensity of biomolecules such as nicotinamide-adenine dinucleotide phosphate NAD(P)H and surrogates of metabolic function such as riboflavin found in fungal spores may vary according to the environmental conditions under which the fungal colonies are growing and also on their concentration at a particular point in time. The decrease in fluorescence with age could also be due to changes in the fluorescent compounds as the culture ages.

Concentration of fungal spores and fragments increased with increased air velocity, but the F/S ratios decreased with increase in air velocity (IV). A decrease in fluorescence per spore was observed when the air velocity was increased (III). The increase in the number of fungal spores and fragments agrees with other studies that have found that increased air velocity augments aerosolization of fungal particles (Górny et al., 2001; Górny et al., 2003; Górny et al., 2002). It is also possible that as larger particles were carried along with the increased air currents in the sampling lines, they impacted on the sides of the walls resulting in the breakage; as posited by Afanou et al. (2014, 2015). Fragments have been proposed to be secondary organic aerosols formed from MVOCs released from fungal growths (secondary formation of aerosol particles) (Górny et al., 2002). If fragment particles form by this mechanism in the presence of ozone, the concentration of fragments should decrease with higher flow rates due to their increased dilution. However, opposite results were obtained in this study, meaning that secondary aerosol

formation was not relevant process for origin of fungal fragments. Instead, fragments are mainly formed through mechanical processes. It has been shown that fungal fragments are aerosolized at low air velocity (Górny, 2004). In the present study, fragments and spore concentrations increased with greater air velocities, however, the spore concentration increased more than the fragment concentration. This explains the decrease in F/S ratio when the air velocity increased. A decrease in fluorescence in response to the increase in air velocity has been postulated to be due to a decrease in relative humidity of the culture causing desiccation stress to the fungal spores (Kanaani et al., 2007). In addition, due to the increased air velocity, larger fungal hyphae are aerosolized together with spores due to increased stress and desiccation of the colony. The desiccation stress and decrease in fluorescence induced by increased air velocity has been attributed to a loss of spore viability (Kanaani et al., 2007).

Since the LIF-devices are not able to detect fluorescence in fragment sized particles (Kanaani et al. 2007; Saari et al., 2014), EDX analysis was employed to distinguish biological fragments from non-biological fragments (IV). The contents of N and P in the fragments which were qualitatively detected were used as indicator elements for distinguishing biological from non-biological fragments (Keszthelyi et al., 1984). It was concluded that most of the fragments originating from fungal growth and only a minority from the growth materials. On the other hand, depending on chemical composition of the growth material, fragments originating from that source could be a potential health hazard.

6.3 SUMMARY AND CONCLUSIONS

The results of the present thesis emphasize the importance of the type of building material and fungal species on the amount of growth measured on material surfaces. In addition, these factors together with air velocity and age of the culture affect the

properties of fungal particles aerosolized from fungal contaminated surfaces. Although so-called green building materials have certain advantages over their non-green counterparts, such as having little or no chemical emissions and being highly recyclable, the two classes of the building materials did not display significant differences in the extents of fungal growth on their surfaces. It is recommended that if one wishes to reduce microbial growth in indoor environments, then materials that are naturally resistant to fungal growth should be preferred. This is because it is the material type, i.e., their nutritional value, chemical composition, and moisture requirements as well as sources of external nutrients potentially affect fungal growth. The above-mentioned factors therefore indicate that when measuring fungal growth, different methods are required because each of the assay methods provides a different perspective of fungal quantification due to its specific responses to various stages of fungal growth and factors that affect growth.

The fluorescence of fungal spores decreases when they are i) grown on poor nutrient substrates, ii) released from older cultures and iii) released in the presence of high air velocities. Since a building has many different materials in its structure and varying airflows passing over different ages of fungal growths at any particular time, it is concluded that fungal concentrations measured with fluorescence-based devices may underestimate spore levels.

The data on measured F/S-ratios in this study revealed a decrease with increased air velocity while the spore concentration increased. This suggests that the conditions under which individuals are exposed to fungal fragments and spores may be different. The study also showed that a fraction of the fragments could be derived from building materials due to biodegradation of these materials when they are subjected to fungal metabolism. The fragments aerosolized from building materials could represent a potential health hazard, depending on the composition of the material.

6.4 FUTURE DIRECTION

All of the measurements in this study were done under laboratory conditions. It is relatively easy to control environmental factors in chambers or laboratory studies, therefore, the results obtained from these studies should be verified in more natural conditions in actual buildings. In addition, larger numbers of building materials should be included in future studies in order to be able to generalize the results to all materials. The results obtained from the SEM/EDX analysis are preliminary and more qualitative in nature. A quantitative approach is recommended when determining the elements in the aerosolized fragments. Furthermore, the characterization of the physicochemical interactions between substrates and fungi needs to be studied. This information could improve our understanding and our ability to predict fungal growth and aerosolization, fluorescence properties and chemical composition of spores and fragments.

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Moisture-damaged building materials promote microbial growth and become sources of microbial contamination in indoor environments. These contaminants, including fungal spores and fragments are associated with adverse health effects among occupants when inhaled. This thesis focused on fungal contamination of building materials evaluating how fungi grow on different building materials and examining their properties when they are aerosolized from the material surfaces in laboratory settings. This information contributes to the understanding of the risk involved in the growth of fungi in indoor environments.

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